

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. **(currently amended)** An isolated nucleic acid molecule comprising:
  - (a) two nucleotide sequences encoding a bacteriophage recombinase ~~function~~;
  - (b) a nucleotide sequence ~~sequences~~ encoding a bacteriophage anti-recombinase ~~function~~;
  - (c) a *Ptac* promoter sequence ~~sequences~~ operably linked to the nucleotide sequences of (a) and (b); and
  - (d) a nucleotide sequence ~~sequences~~ encoding LacI operably linked to its native promoter.
2. **(currently amended)** The nucleic acid molecule of claim 1, further comprising at least one origin of replication sequence ~~sequences~~ which confers ~~confer~~ low copy number on a vector comprising the nucleic acid molecule.
3. **(original)** The nucleic acid molecule of claim 2, wherein the origin of replication is temperature sensitive.
4. **(currently amended)** An isolated nucleic acid molecule comprising:
  - (a) two nucleotide sequences encoding bacteriophage  $\lambda$  Red recombinase ~~function~~;
  - (b) a nucleotide sequences encoding bacteriophage  $\lambda$  anti-RecBCD ~~function~~;
  - (c) a *Ptac* promoter sequence ~~sequences~~ operably linked to the nucleotide sequences of (a) and (b); and
  - (d) a nucleotide sequence ~~sequences~~ encoding LacI operably linked to its native promoter.
5. **(currently amended)** The nucleic acid molecule of claim 4, further comprising at least one origin of replication sequence ~~sequences~~ which confers ~~confer~~ low copy number on a vector comprising the nucleic acid molecule.
6. **(original)** The nucleic acid molecule of claim 5, wherein the origin of replication is temperature sensitive.

7. **(currently amended)** The nucleic acid molecule of any one of claims 4-6, wherein the nucleotide sequences encoding bacteriophage  $\lambda$  Red recombinase ~~function~~ comprises ~~comprises~~  $\lambda$  *exo* and *bet* sequences.

8. **(currently amended)** The nucleic acid molecule of any one of claims 4-6, wherein the nucleotide sequences encoding  $\lambda$  anti-RecBCD ~~function~~ comprises ~~comprises~~  $\lambda$  *gam* sequences.

9. **(currently amended)** A vector comprising:

- (a) two nucleotide sequences encoding a bacteriophage recombinase ~~function~~;
- (b) a nucleotide sequence ~~sequences~~ encoding a bacteriophage anti-recombinase ~~function~~;
- (c) a *Ptac* promoter sequence ~~sequences~~ operably linked to the nucleotide sequences of (a) and (b);
- (d) a nucleotide sequence ~~sequences~~ encoding LacI operably linked to its native promoter; and
- (e) at least one origin of replication sequence ~~sequences~~ which confers ~~confers~~ low copy number on the vector.

10. **(currently amended)** The vector of claim 9, wherein the origin of replication sequence is ~~sequences are~~ temperature sensitive.

11. **(currently amended)** A vector comprising:

- (a) two nucleotide sequences encoding bacteriophage  $\lambda$  Red recombinase ~~function~~;
- (b) a nucleotide sequence ~~sequences~~ encoding bacteriophage  $\lambda$  anti-RecBCD ~~function~~;
- (c) a *Ptac* promoter sequence ~~sequences~~ operably linked to the nucleotide sequences of (a) and (b); and
- (d) a nucleotide sequence ~~sequences~~ encoding LacI; and
- (e) at least one origin of replication sequence ~~sequences~~ which confers ~~confers~~ low copy number on the vector.

12. **(currently amended)** The vector of claim 11, wherein the origin of replication sequence is ~~sequences are~~ temperature sensitive.

13. **(currently amended)** The vector of claim 12, wherein the nucleotide sequence ~~sequences~~ encoding bacteriophage  $\lambda$  Red recombinase ~~function~~ comprises ~~comprises~~  $\lambda$  *exo* and *bet* sequences.

14. **(currently amended)** The vector of claim 12, wherein the nucleotide ~~sequences~~ sequence encoding  $\lambda$  anti-RecBCD ~~function~~ comprises a ~~comprises~~  $\lambda$  *gam* sequence ~~sequences~~.

15. **(original)** A recombinant organism comprising the vector of any one of claims 9-14.

16. **(original)** The recombinant organism of claim 15, which is a bacteria.

17. **(original)** The recombinant organism of claim 16 which is of the genus *Escherichia*.

18. **(original)** The recombinant organism of claim 17, which is *Escherichia coli*.

19. **(original)** The recombinant organism of claim 18, which is *Escherichia coli* K12.

20. **(original)** The recombinant organism of claim 16 which is a pathogenic species.

21. **(original)** The recombinant organism of claim 20 which is a pathogenic *Escherichia coli*.

22. **(original)** The recombinant organism of claim 21 which is enterohemorrhagic *E. coli* (EHEC) or enteropathogenic *E. coli* (EPEC).

23. **(original)** The recombinant organism of claim 15 which is of the genus *Pseudomonas*.

24. **(original)** The recombinant organism of claim 23, which is *Pseudomonas aeruginosa*.

25. **(original)** The recombinant organism of claim 15 which is of the genus *Mycobacterium*.

26. **(original)** The recombinant organism of claim 25, which is *Mycobacterium tuberculosis*.

27. **(withdrawn)** A method of promoting efficient recombination of genetic material in a microorganism comprising use of the vector of any one of claims 9-14.

28. **(withdrawn)** The method of claim 27, wherein the genetic material is endogenous.

29. **(withdrawn)** The method of claim 27, wherein the genetic material is exogenous

30. **(withdrawn)** The method of claim 27, wherein the genetic material is derived from a prokaryote.

31. **(withdrawn)** The method of claim 27, wherein the genetic material is derived from a eukaryote.

32. **(withdrawn)** The method of claim 27, wherein the genetic material is derived from a fungi.

33. **(withdrawn)** A method for determining whether a bacterial gene is a potential drug target comprising:

- (a) introducing a test construct into the microorganism of claim 15, wherein the test construct comprises an integrating segment flanked by recombination segments; wherein the recombination segments are homologous to the bacterial gene or surrounding sequences; and
- (b) culturing the microorganism under conditions such that recombination between the test construct and the bacterial gene occurs; and
- (c) assaying the microorganism for growth and/or pathogenicity or an indicator thereof,

whereby a change in growth and/or pathogenicity or an indicator thereof identifies the bacterial gene as a potential drug target.

34. **(withdrawn)** The method of claim 33, wherein the bacterial gene is chromosomal.

35. **(withdrawn)** The method of claim 33, wherein the bacterial gene is present on an endogenous plasmid.

36. **(withdrawn)** The method of claim 33, wherein the integrating segment comprises nucleotide sequences encoding a selectable marker.

37. **(withdrawn)** The method of claim 36, wherein the selectable marker is selected from the group consisting of ampicillin (Amp), kanamycin (Kan), tetracycline (Tat), and  $\beta$ -glycosidase ( $\beta$ -gal).

38. **(withdrawn)** A method of cloning a potential vaccine antigen comprising:

- (a) introducing a substrate into the microorganism of claim 15, wherein the substrate comprises recombination segments comprising nucleotide sequences homologous to a potential vaccine antigen gene or surrounding native sequences; and
- (b) culturing the microorganism under conditions such that recombination between the substrate and the vaccine-antigen gene sequences or surrounding native sequences occurs;

such that *in vivo* cloning of the vaccine antigen occurs.

39. **(withdrawn)** A vaccine comprising an antigen identified according to the method of claim 38.

40. **(withdrawn)** Use of the recombinant organism of claim 20 in the manufacture of a vaccine.

41. **(withdrawn)** A method of producing an attenuated pathogenic microorganism, comprising:

- (a) introducing a vector of any one of claims 9-14 into a pathogenic microorganism;
- (b) introducing a substrate into the pathogenic microorganism, wherein the substrate comprises recombination segments comprising nucleotide

sequences homologous to a gene required for pathogenicity or surrounding native sequences; and

- (c) culturing the microorganism under conditions such that recombination between the substrate and the gene sequences or surrounding native sequences occurs;

such that the gene required for pathogenicity is mutated, thereby producing an attenuated pathogenic microorganism.

42. **(withdrawn)** An attenuated pathogenic microorganism produced according to the method of claim 41.

43. **(withdrawn)** A vaccine comprising an attenuated pathogenic microorganism of claim 42.